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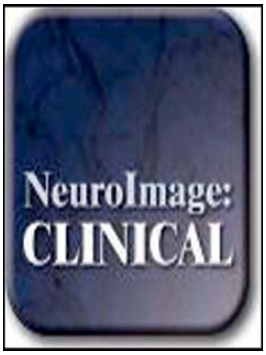


# Fronto-striatal glutamate in children with Tourette's Disorder and Attention-deficit/Hyperactivity Disorder

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**Title:** Fronto-striatal glutamate in children with Tourette's Disorder and Attention-deficit/Hyperactivity Disorder

**Running title:** Fronto-striatal glutamate in TD and ADHD

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**Presentation information**

Data contributing to this study was presented as a poster at the annual meeting of the International Society for Research on Impulsivity, 2015, Amsterdam, The Netherlands and European Society for the Study of Tourette Syndrome meeting, 2016, Warsaw, Poland.

**Key words:** Tourette Syndrome, ADHD, glutamate, fronto-striatal circuit, MRS

**Abstract**

**Objective:** Both Tourette's Disorder (TD) and Attention-Deficit/Hyperactivity Disorder (ADHD) have been related to abnormalities in glutamatergic neurochemistry in the fronto-striatal circuitry. TD and ADHD often co-occur and the neural underpinnings of this co-occurrence have been insufficiently investigated in prior studies.

**Method:** We used proton magnetic resonance spectroscopy (1H-MRS) in children between 8-12 years of age (TD n=15, ADHD n=39, TD+ADHD n=29, and healthy controls n=53) as an *in vivo* method of evaluating glutamate concentrations in the fronto-striatal circuit. Spectra were collected on a 3 Tesla Siemens scanner from two voxels in each participant: the anterior cingulate cortex (ACC) and the left dorsal striatum. LC-model was used to process spectra and generate glutamate concentrations in institutional units. A one-way analysis of variance was performed to determine significant effects of diagnostic group on glutamate concentrations.

**Results:** We did not find any group differences in glutamate concentrations in either the ACC ($F_{(3,132)}$=0.97, $p$=0.41) or striatum ($F_{(3,121)}$=0.59, $p$=0.62). Furthermore, variation in glutamate concentration in these regions was unrelated to age, sex, medication use, IQ, tic, or ADHD severity. Obsessive-compulsive (OC) symptoms were positively correlated with ACC glutamate concentration within the participants with TD (rho=0.35, $p_{uncorrected}$=0.02)**.**

**Conclusion:** We found no evidence for glutamatergic neuropathology in TD or ADHD within the fronto-striatal circuits. However, the correlation of OC-symptoms with ACC glutamate concentrations suggests that altered glutamatergic transmission is involved in OC-symptoms within TD, but this needs further investigation.

## 1. Introduction

Tourette's Disorder (TD) and Attention-Deficit/Hyperactivity Disorder (ADHD) are early onset neurodevelopmental disorders affecting approximately 1%[1] and 5%[2] of children and adolescents, respectively. While TD is characterized by the presence of motor and vocal tics[3] there are also psychiatric comorbidities present in up to 86% of those with TD during their lifetime.[4] ADHD is the most common, occurring in approximately 40% of cases[5] and even more TD patients have ADHD symptoms that do not meet the threshold for diagnosis.[6] Conversely, the presence of tics within patients with ADHD has been estimated at 20%.[7] ADHD itself is characterized by age inappropriate inattention and/or hyperactivity/impulsivity leading to impaired functioning.[3]

Both disorders have been associated with abnormalities in fronto-striatal circuits,[8,9] although the overlap in conditions has confounded research to date. Structural and functional neuroimaging studies have reported alterations in the caudate nuclei, putamen, and anterior cingulate cortex (ACC) in TD[10,11] and ADHD[12–14] relative to controls, although not always consistently. It has been proposed that excitatory abnormalities in the striatum cause erroneous inhibition of neurons in the globus pallidus (GP) internus, which in turn leads to disinhibition of prefrontal neurons which results in tic phenomena.[15] These striatal abnormalities may also underlie the high rate of comorbidity with other disorders, like ADHD and obsessive compulsive disorder (OCD)[4] due to the aberrant integrative interplay of different fronto-striatal circuits including connections with the ACC.[15,16]Dopamine dysfunction within the fronto-striatal circuit has long been considered the primary cause of tics[17] and has been related to difficulties with attention and impulsivity.[18] However, as glutamatergic, GABAergic, serotonergic, cholinergic, and opioid as well as dopaminergic systems all operate within the fronto-striatal circuits it is plausible that multiple neurotransmitter systems may be involved in TD and ADHD.[19] Glutamate is the primary

excitatory neurotransmitter found in the brain,[20,21] essential in fronto-striatal transmission and often co-transmitted with dopamine.[22] Post-mortem analysis of a small number of brains from people who had TD corroborate the view that glutamate is involved in TD as reduced levels of glutamate were seen in the GP and substantia nigra of the TD brains compared to control brains.[23]

Additional insights into the underlying neurobiology of TD and ADHD can be found by investigating brain neurochemistry. This can be achieved by using proton magnetic resonance spectroscopy (1H-MRS) which allows for non-invasive *in vivo* quantification of specific neurometabolites. There have been just four MRS studies of TD to date, three of which focused on GABA concentrations either in the primary and secondary motor areas[24] or the sensory motor cortex.[25,26] DeVito and colleagues[27] on the other hand investigated multiple neurochemicals including Glx, the combined signal from glutamatergic compounds (glutamate+glutamine), within multiple regions; premotor cortex, caudate nucleus, putamen and thalamus with a 3 Tesla scanner in a sample of 25 (male only) children and adolescents with TD in comparison to controls. No group differences were seen in Glx in any of the regions. Within the putamen lower creatine (Cre) levels bilaterally and lower N-acetyl aspartate and choline in the left putamen were found. Reduced Cre bilaterally in the caudate nucleus was also seen but this did not reach significance.

Many more MRS studies of disorders related to TD, such as ADHD and OCD, have been conducted. For a review of these studies in ADHD, OCD and autism spectrum disorder (ASD) see Naaijen and colleagues.[28] However, findings were inconsistent, plagued by heterogeneous methodologies, sample selection (i.e., child or adult, inclusion or exclusion of comorbidities), voxel placement, and often inadequate field strengths to distinguish glutamate from glutamine.[28] Despite these issues the review tentatively summarized increased striatal

Glx levels are associated with both ADHD and OCD and increased ACC Glx levels with pediatric ADHD.

In the current study we assessed a large number of children between the ages of 8-12 years which allowed us to focus on a group where tics are most frequently observed and not limit ourselves to the subset of patients whose tics persist into adulthood.[29] Furthermore we directly addressed the confounds of comorbidity rampant in previous studies by including a TD+ADHD group in addition to ADHD, TD, and healthy control (HC) groups. Based on previous findings in childhood ADHD,[28] we expected increased glutamate concentrations in both regions of interest. This is the first study to investigate fronto-striatal glutamate in children with TD. Given the theory that excitatory abnormalities in the striatum result in tics, we expected to observe raised glutamate in the striatum of TD patients.

## 2. Method

### 2.1 Participants

Participants with TD and/or ADHD: TD with/without ADHD n=60, ADHD without TD n=60 were recruited via child and adolescent psychiatry departments and patient associations throughout the Netherlands, while healthy controls (HC; n=60) were found mainly through schools. The final numbers included for analysis (i.e. with usable data) can be found in section 3.1 and Table 1 (n=136 for the ACC and n=125 for the striatum). Written informed consent was provided by the parents/guardians of all participants and written assent was also given by participants who were 12 years of age. This study was approved by the regional ethics board (CMO Regio Arnhem-Nijmegen, numbers: NL42004.091.12 & NL48377.091.14).

Inclusion criteria for all participants included being aged 8-12 years, IQ>70, Caucasian decent, no previous head injuries or neurological disorders, no contra indications for MRI assessment, and no major physical illness. Inclusion criteria for ADHD and TD were meeting DSM-5 criteria for these disorders. Those with sub-threshold ADHD (Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS[30]) score of 4 or 5 on either subscale) were also included. Persistent Motor or Vocal Tic Disorder (Motor type) was also allowed for the TD group. Common psychiatric comorbidities like oppositional defiant disorder were not excluded. Within the TD group, ADHD, and OCD were not excluded, while in the ADHD group those with tics and/or OCD were excluded. Within the HC group no psychiatric disorders were allowed, as determined by screening questionnaires (Child behavior checklist [CBCL] and Teacher Report Form [TRF][31]). Subjects were divided into four groups; HC, TD, ADHD, and TD+ADHD, see Table 1 for demographics. Participants were required to refrain from consuming caffeine on the day of testing. Medications for tics

were continued as normal while stimulant medication was withheld for 48 hours before testing.

### 2.2 Phenotypic information

TD diagnosis was confirmed, and tic severity rated, by diagnostic interview with parent(s) and child present using the Yale Global Tic Severity Scale (YGTSS[32]). To determine the presence of ADHD and/or other psychiatric disorders the K-SADS[30] interview was administered to the parent(s). All interviews were conducted by experienced researchers who were trained and overseen by a child- and adolescent psychiatrist (JKB). The screening module was used, followed if needed by disorder-specific modules. If participants screened positive for possible OCD the Children's Yale-Brown Obsessive Compulsive Scale (CY-BOCS[33]) was administered with both parent(s) and child present. The CY-BOCS interview was conducted with each participant of the TD group due to the relatedness of symptoms and common co-occurance of OCD and TD.

Full-scale IQ was estimated by four subtests of the Wechsler Intelligence Scale for Children-III (WISC-III[34]): Vocabulary, Similarities, Block design, and Picture completion. Questionnaires were further used to assess phenotypic traits. The Conners' Parent Rating Scale – Revised Long version (CPRS-RL[35]) was used to rate ADHD severity. Additional questionnaires were used to assess the presence of autistic symptoms and compulsive behaviors; the Children's Social Behavioral Questionnaire (CSBQ[36]) and Repetitive Behavior Scale (RBS-R[37]). Information about medication history was gathered from parental report which has previously been shown to correlate well with pharmacy records.[38] Interviews on psychiatric symptoms were conducted about an unmedicated period.

### 2.3 T1-weighted MRI acquisition

All MRI datasets were acquired on the same 3T Siemens Prisma (Siemens, Erlangen, Germany) scanner located in the Donders Institute for Brain, Cognition and Behaviour, Nijmegen, the Netherlands. T1-weighted anatomical images were acquired with a transversal, 3D magnetization prepared rapid gradient echo (MPRAGE) parallel imaging sequence with the following parameters: TR = 2300 ms, TE = 2.98 ms, TI = 900 ms, FoV = 256 mm, slice thickness = 1.20 mm, Flip angle 9 degrees, in plane resolution = 1.0×1.0 mm, acceleration factor = 2, acquisition time = 5:30 min. Each participant also had their head stabilized with cushions during scanning and had a piece of tape across their foreheads to help awareness of possible movement while scanning. All participants were first familiarized with the MRI procedure in a mock scanner where the importance of lying still was explained.

### 2.4 MRS acquisition

Proton spectra were acquired using a point resolved spectroscopy (PRESS) sequence in two regions of interest, with chemically selective suppression (CHESS) water suppression.[39] A single 8 $cm^3$ voxel was centered on the midline covering the pregenual ACC anterior and slightly superior to the genu of the corpus callosum (TR = 3000 ms, TE = 30 ms, number of averages = 96, bandwidth = 5 kHz, number of points = 4096). A second, similar voxel was located in the left dorsal striatum covering the caudate nucleus and putamen. Unsuppressed water reference spectra (16 averages) were also acquired as part of the standard acquisition. See Figure 1 for location of the voxels and an example spectrum. Both voxels were placed to include a maximum amount of grey matter and a minimum amount of cerebrospinal fluid (CSF). T1-weighted images were used for voxel placement and later for tissue segmentation during processing. Acquisition time was six minutes per voxel.

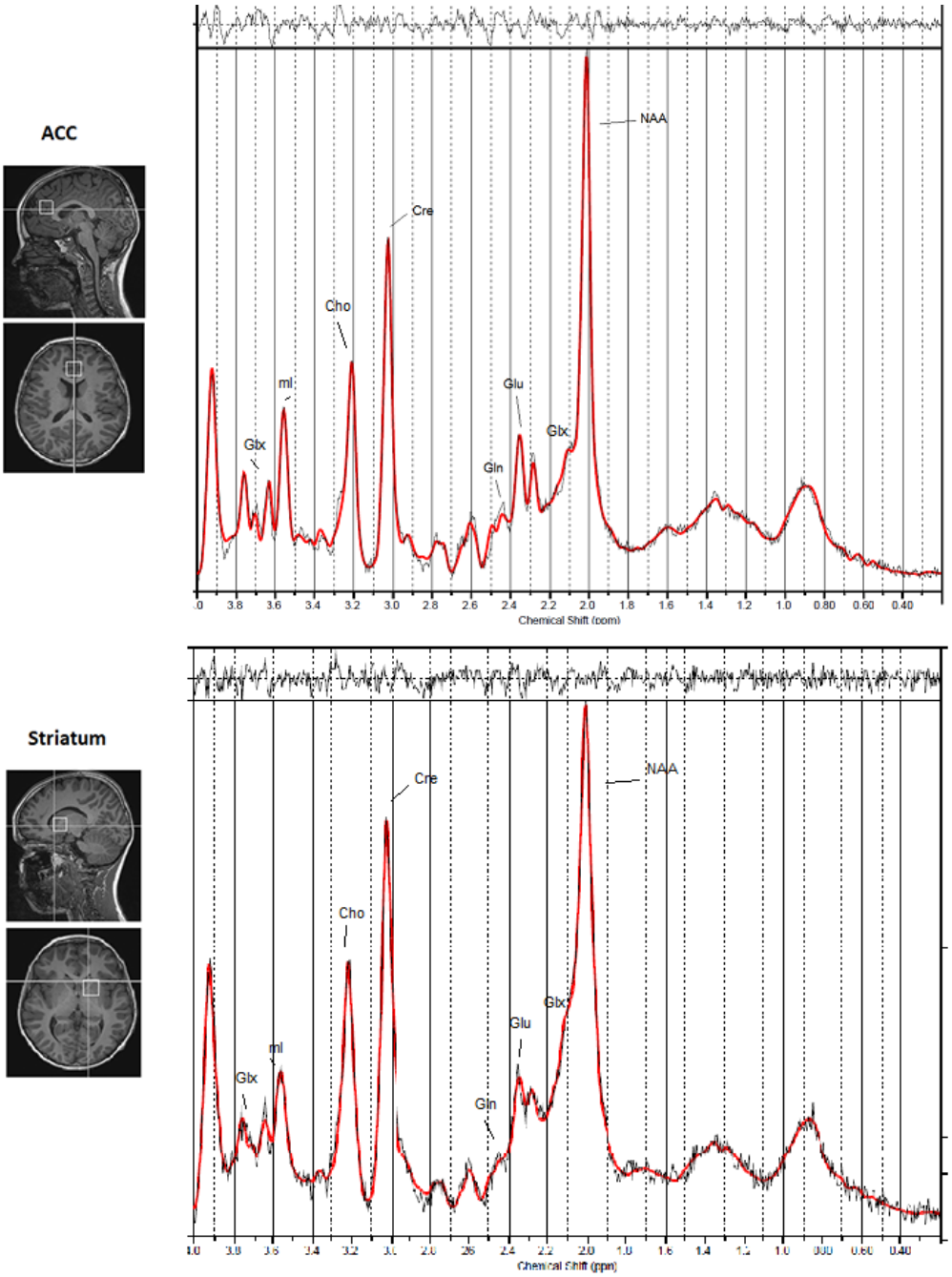


Figure 1. Location of the two voxels are shown on a T1-weighted anatomical image for the pregenual ACC including an example spectrum (top) and the left dorsal striatum including an example spectrum (bottom). Peaks corresponding to individual metabolites are highlighted. The thin black line represents the frequency-domain data, the red line is the LCModel fit. In the top panel the residuals are plotted (the data minus the fit). Cho, Choline; Cre, creatine; Gln, glutamine; Glu, glutamate; Glx, Glu+Gln; mI, myo-inositol; NAA, N-Acetylaspartate.

### 2.5 Processing

LCModel, version V6.03-0I,[40,41] was used to conduct spectral analysis. LCModel uses a linear combination of simulated or *in vitro* metabolite solution spectra as a reference to identify and quantify the major resonance of *in vivo* spectra.

Water referenced metabolite concentrations were automatically calculated in institutional units (i.u.). Institutional units are presented since we did not correct for the $T_1$ and $T_2$ relaxation times of the metabolites or the $T_1$ relaxation time of water. The $T_2$ of tissue water was corrected for assuming the signal had decayed by 30% at the echo time.[42] No correction was made for the $T_2$ decay of metabolites. In addition, there are other scanner-dependent factors that can affect the absolute scaling (e.g., details of coil combination), such that metabolite concentrations measured in i.u. are preferred over attempting to scale to absolute concentrations in millimolar (mM). The unified segmentation procedure within the VBM8 toolbox of SPM8 (Statistical Parametric Mapping release 8, London, UK) was used to process the T1 images and produce grey matter (GM), white matter (WM), and CSF probability maps. Spectroscopy voxels were mapped onto these maps to provide the partial volume of GM, WM, and CSF within each spectroscopy voxel ($f_{GM}$, $f_{WM}$, and $f_{CSF}$), and to allow for group comparisons of the placement of the spectroscopy voxels. Additionally, to correct for differing amounts of water in each tissue and for partial volume effects we corrected individual metabolite concentrations for water concentrations with the following formula[43]:

$$\text{Metabolite}_{\text{corrected}} = \text{Metabolite}_{\text{Raw}} * ((43300 * f_{GM} + 35880 * f_{WM} + 55556 * f_{CSF})/35880) * (1/(1 - f_{CSF}))$$

where 43300, 35880, and 55556 are the water concentrations in mM for GM, WM, and CSF,[44] respectively, as described by the LCModel manual.[45] These concentrations correspond to 77.9, 64.4 and 100%, respectively, assuming that CSF is pure water.[44] This includes a correction for the fraction of the MRS voxel occupied by CSF, along with corrections for the water concentration in each of the tissue types. The factor 35880 in the denominator is included since the initial LCModel analysis was carried out assuming the voxel was pure WM.

### 2.6 Quality control

Statistical analyses were restricted to spectra with linewidth (full-width at half-maximum; FWHM) ≤0.1 ppm, Cramér-Rao lower bounds (CRLB) ≤20%, signal to noise ratio ≥5 or corrected glutamate concentrations less than two standard deviations from the mean. Furthermore, anatomical scan quality was visually checked as these were used for voxel placement and tissue segmentation. Fifteen participants were excluded from both analyses due to poor structural scan quality. A further seven spectra from the ACC (n=136) and 20 spectra from the striatal analyses (n=125) were excluded based on spectral quality. In addition to the exclusion of those with ≥20% CRLB values, we investigated group differences in CRLBs to verify that possible differences in glutamate levels were not due to differences in CRLBs.[46]

### 2.7 Statistics

Statistical analyses were conducted with the R statistical program.[47] Differences between the four groups in categorical measures were tested with Pearson's chi-squared tests. Group differences in continuous measures were assessed with a one way analysis of variance (ANOVA) or Welch Two Sample t-test if assumptions of homogeneity of variance and normality of distributions were met ($p$>0.05 in Bartlett test of homogeneity of variance and Shapiro-Wilk normality test). If these assumptions were violated a non-parametric Kruskal-

Wallis rank sum test was used. ADHD severity was only tested between the ADHD and TD+ADHD group. Similarly measures related to tics and OC-symptoms were only tested between the TD and TD+ADHD groups.

Group differences in voxel tissue composition and spectral quality were assessed with a ANOVA. Group differences in glutamate were analyzed first with age and sex included as covariates. These covariates were removed from analysis as they did not significantly contribute to the model. This resulted in the use of an ANOVA followed if appropriate by Bonferroni corrected *post-hoc* pairwise t-tests. The influence of IQ, ADHD severity, ASD symptoms, repetitive and compulsive behaviors, and medication status were also examined by inclusion in an ANCOVA. Correlations between glutamate levels and phenotypic measures of those with TD (tic severity, age of onset and duration since tic onset, and OC-symptoms) were assessed with Pearson's correlation tests if normally distributed or Spearman's rank correlation rho test if not. Tests were then Bonferroni corrected for multiple comparisons. In the supplementary material we added the same analysis for Glx.

## 3. Results

### 3.1 Demographics

MR spectra were acquired for a total of 162 of the original 180 participants. Three participants from the ADHD group were found not to have ADHD (K-SADS <4 in both subscales) and one presented with tics but did not meet criteria for TD or Chronic Motor Tic disorder (CMT). These four participants were thereafter excluded from analysis. Due to spectral or segmentation quality concerns 22 spectra were excluded from the ACC analysis (n=136) and 35 from the striatal analysis (n=125). The TD group was subdivided into those that also had ADHD (TD+ADHD; n=29, 27 for the ACC and striatal analyses, respectively) and those that did not (TD; n=15, 17 for the ACC and striatal analyses, respectively). Participants with sub-threshold ADHD were included in either the ADHD group or the TD+ADHD group if comorbid with TD. Details of the groups used for analysis of the ACC are reported in Table 1 (n=136).

Table 1. Demographic description of participants included in the ACC analysis

| | Control | TD | ADHD | TD+ADHD | Test statistic | *p*-value |
|---|---|---|---|---|---|---|
| N | 53 | 15 | 39 | 29 | | |
| Age years, mean(SD) | 10.0(1.0) | 10.4(1.2) | 10.7(1.2) | 10.7(1.6) | K-W $\chi^2$=2.98 | 0.40 |
| Sex, m/f | 38/15 | 13/2 | 21/18 | 25/4 | $\chi^2$=10.70 | 0.01* |
| [a]IQ, mean(SD) Range | 109(11), 86 – 133 | 105(12), 81 – 126 | 103(13), 71 – 137 | 106(11), 85 – 124 | F=2.47 | 0.06 |
| Handed, r/l | 48/5 | 14/1 | 36/3 | 25/4 | $\chi^2$=0.90 | 0.82 |
| [b]ADHD severity, mean(SD) | T=45.5(4.9) | T=51.7(7.0) | T=71.9(9.7) | T=68.7(9.1) | *t*=1.37 | 0.18† |
| | I=45.5(6.0) | I=50.9(8.2) | I=69.3(10.0) | I=65.2(9.8) | *t*=1.69 | 0.10† |
| | H=46.1(3.7) | H=52.2(8.5) | H=71.2(11.1) | H=70.0(10.4) | *t*=-0.44 | 0.66† |
| CSBQ core autism score, mean(SD) | 1.42(1.89) | 9.53(10.58) | 12.46(7.78) | 17.66(9.40) | | |
| RBS compulsivity score, mean(SD) | 0.09(0.35) | 1.67(1.59) | 0.69(1.24) | 2.55(3.00) | | |
| [c]Tic dx, n | - | TD=14 CMT=1 | - | TD=29 | | |
| [c]Tic severity, mean(SD) | - | T=20.4(7.9) M=13.6(4.3) V=6.8(4.7) | - | T=20.8(9.2) M=13.1(5.5) V=7.7(5.7) | *t*=-0.13 *t*=0.33 K-W $\chi^2$=0.22 | 0.89 0.75 0.64 |
| [c]Age tic onset years, mean(SD) | - | 5.3(1.7) | - | 5.7(1.7) | *t*=-0.75 | 0.46 |
| [c]Duration since tic onset years, mean(SD) | - | 5.0(1.8) | - | 5.0(2.0) | *t*=0.06 | 0.95 |
| [d]OCD dx, n | - | 3 | - | 6 | | |
| [d]OC-symptoms, mean(SD) | - | 8.47(8.6) | - | 6.75(8.4) | K-W $\chi^2$ =0.78 | 0.38 |
| [e]Medication | | | | | | |
| Stimulant | | 0 | 24 | 9 | | |
| Strattera | - | 0 | 1 | 0 | | |
| Antipsychotic | | 2 | 1 | 7 | | |
| Clonidine | | 0 | 0 | 2 | | |

[a]IQ estimated from a subtest of the Wechsler Intelligence Scale for Children-III (WISC-III[34]) rating. [b]ADHD severity ratings reflect T-scores from the Conners' Parent Rating Scale –

Revised Long version.[35] [c]Tic diagnosis and severity were determined and rated with the Yale Global Tic Severity Scale.[32] Tic severity is reported excluding impairment score. [d]OCD diagnosis was determined as a total-score of ≥16 on the Children's Yale-Brown Obsessive Compulsive Scale.[33] [e]Current medication status, determined from parental report of current and previous medication use. †Statistics refer to an ADHD versus TD+ADHD contrast. *$p$<0.05, **$p$<0.01. ADHD, Attention-Deficit/Hyperactivity Disorder; CSBQ, Children's Social Behavioral Questionnaire; CMT, Chronic Motor Tic; dx, diagnosis; H, hyperactive; I, inattentive; C, combined; K-W, Kruskal-Wallis; M, motor; m/f, male/female; OCD, Obsessive Compulsive Disorder; r/l, right/left; RBS, Repetitive Behavior Scale; SD, standard deviation; *t*, Welch Two sample t-test; T, total; TD, Tourette's Disorder; V, vocal.

Analysis of the striatum included fewer participants (n=125) due to exclusion based on spectral quality (n=22). This did not significantly alter the demographic distributions between groups (n=48, 17, 33, and 27 for the HC, TD, ADHD, and TD+ADHD groups, respectively) regarding age (K-W $\chi^2$=1.64, $p$=0.65), sex ($\chi^2$=8.09, $p$=0.04), IQ ($F_{(3,117)}$=2.25, $p$=0.09) and handedness ($\chi^2$=0.82, $p$=0.84). ADHD severity between the ADHD and TD+ADHD groups differed slightly but not significantly with respect to total and inattentive scores ($t$=1.90, $p$=0.06; $t$=1.84, $p$=0.07; $t$=0.99, $p$=0.33 for total, inattentive, and hyperactive CPRS scores, respectively) while tic severity ($t$=0.31, $p$=0.76; $t$=-0.54, $p$=0.59; K-W $\chi^2$=~0, $p$=0.99 for total, motor, and vocal YGTSS scores respectively) and OC-symptoms (K-W $\chi^2$=1.52, $p$=0.22) remained similar between the TD and TD+ADHD groups.

Age of tic onset ($t$=-0.51, $p$=0.62) and duration since tic onset ($t$=-0.06, $p$=0.95) did not differ significantly between the TD and the TD+ADHD group. For both analyses sex was not balanced between groups, mainly due to a low number of girls with TD having been included. This reflects the proportionately fewer girls affected by TD compared to boys.[48] Sex was included in the model to account for this imbalance, however, it was found not to affect the model significantly and was therefore subsequently removed.

### 3.2 Spectral quality

Groups did not differ significantly in mean voxel percentage GM, WM or CSF in either ACC ($F_{(3,132)}$=0.30, $p$=0.83, $F_{(3,132)}$=0.61, $p$=0.61 and $F_{(3,132)}$=0.26, $p$=0.85, respectively) or striatum ($F_{(3,121)}$=1.77, $p$=0.16, $F_{(3,121)}$=1.77, $p$=0.16 and $F_{(3,121)}$=1.73, $p$=0.16, respectively). In the ACC voxel across all groups the tissue percentages were: GM 70 (7)%, WM 11 (2)% and CSF 18 (7)%. For the striatal voxel these were GM 58 (7)%, WM 42 (7)% and CSF 1 (1)%.

To verify that the spectral quality did not differ between the groups, we compared the CRLB estimated standard deviations in both of the voxels, using a one-way ANOVA across the four groups. CRLB's did not differ between groups in the ACC ($F_{(3,132)}$=1.35, $p$=0.26) or the striatum ($F_{(3,121)}$=0.37, $p$=0.77). Furthermore all CRLB's were in the range 3-7% SD, all SNR were > 20 and all FWHM were in the range of 0.02-0.09 reflecting overall good quality of the ACC spectrum in all four groups. For the striatum, CRLB's were in the range 5-17%, SNR were > 11 and FWHM were in the range of 0.04-0.09.

### 3.3 ACC

Age and sex had no significant influence on the ANCOVA model and were subsequently excluded. There was no group difference in corrected glutamate levels ANOVA ($F_{(3, 132)}$=0.97, $p$=0.41, Figure 2). There was no influence of IQ ($p$=0.61), total CPRS ADHD severity T-score ($p$=0.56), inattentive CPRS T-score ($p$=0.70), hyperactive CPRS T-score ($p$=0.48), CSBQ core autism symptom-score ($p$=0.64) or RBS compulsivity score ($p$=0.92). Current medication use showed no significant effect on glutamate levels when any current medication ($p$=0.65), current stimulant medication ($p$=0.28) or current antipsychotic medication ($p$=0.56) were investigated.

There were no correlations in those with tics between corrected glutamate concentrations and tic severity (total $p$=0.77, motor $p$=0.40, vocal $p$=0.53), duration since ($p$=0.38) or age of

onset ($p$=0.16). A significant positive correlation (rho=0.35) between ACC glutamate and CY-BOCS total score ($p$=0.02) was found. This correlation was also present for the obsessions (rho=0.30, $p$=0.045) and compulsions (rho=0.31, $p$=0.04) severity scales separately. However, the two subscales were highly correlated (rho=0.55, $p$<0.001). However, none of the correlations with glutamate concentration survived correction for multiple comparisons (12 correlation analyses). The analysis was limited to the participants that were administered the CY-BOCS (n=44, all with TD).

### 3.4 Striatum

Similarly for the striatal analysis there were no significant effects of age or sex on glutamate levels. Group analysis revealed no difference in striatal corrected glutamate levels ($F_{(3, 121)}$=0.59, $p$=0.62, Figure 2). Again there was no influence of IQ ($p$=0.63), total CPRS ADHD severity T-score ($p$=0.78), inattentive CPRS T-score ($p$=0.80), hyperactive CPRS T-score ($p$=0.75), CSBQ core autism symptom score ($p$=0.37), RBS compulsivity score ($p$=0.25) or current medication use (any $p$=0.73, stimulant $p$=0.80 or antipsychotic $p$=0.06). There were no correlations between corrected glutamate concentrations and tic severity (total $p$=0.34, motor $p$=0.46 vocal $p$=0.22), duration since ($p$=0.19) or age of onset ($p$=0.08). There was no association between glutamate and CY-BOCS total score (rho=-0.25, $p$=0.11).

### 3.5 Glx

There were also no group differences in Glx concentration in either the ACC or striatum (see supplementary information for details).

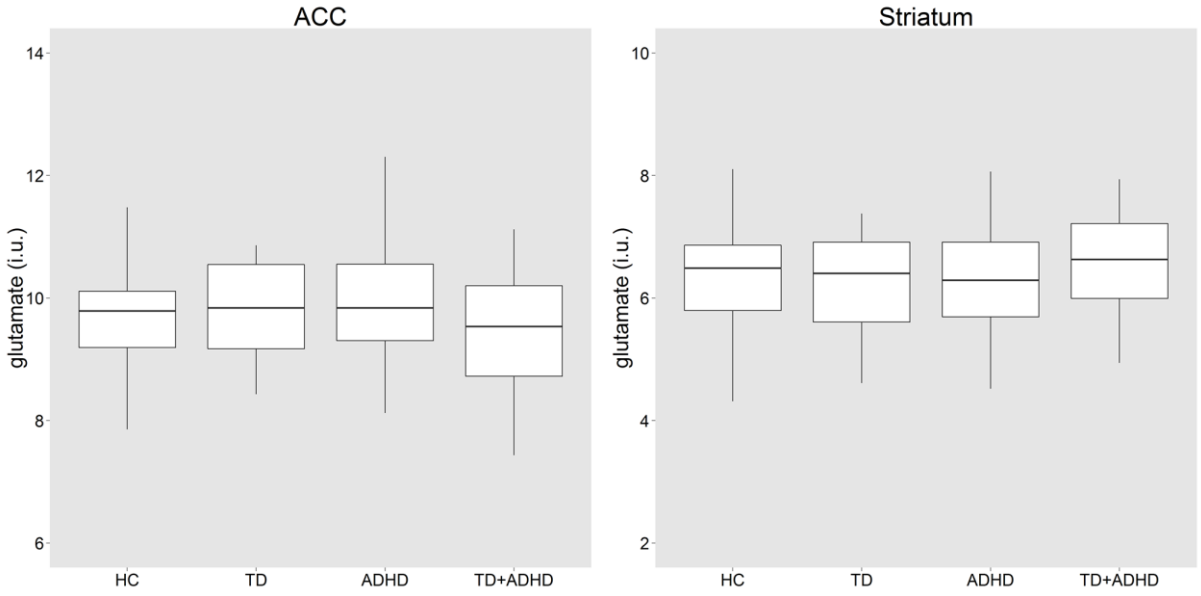


Figure 2. Boxplots of glutamate concentrations per group in the ACC and striatum. No group differences in glutamate levels were seen. ADHD, attention-deficit/hyperactivity disorder; HC, healthy controls; i.u., Institutional Units; TD, Tourette's disorder; TD+ADHD, Tourette's disorder and comorbid attention-deficit/hyperactivity disorder.

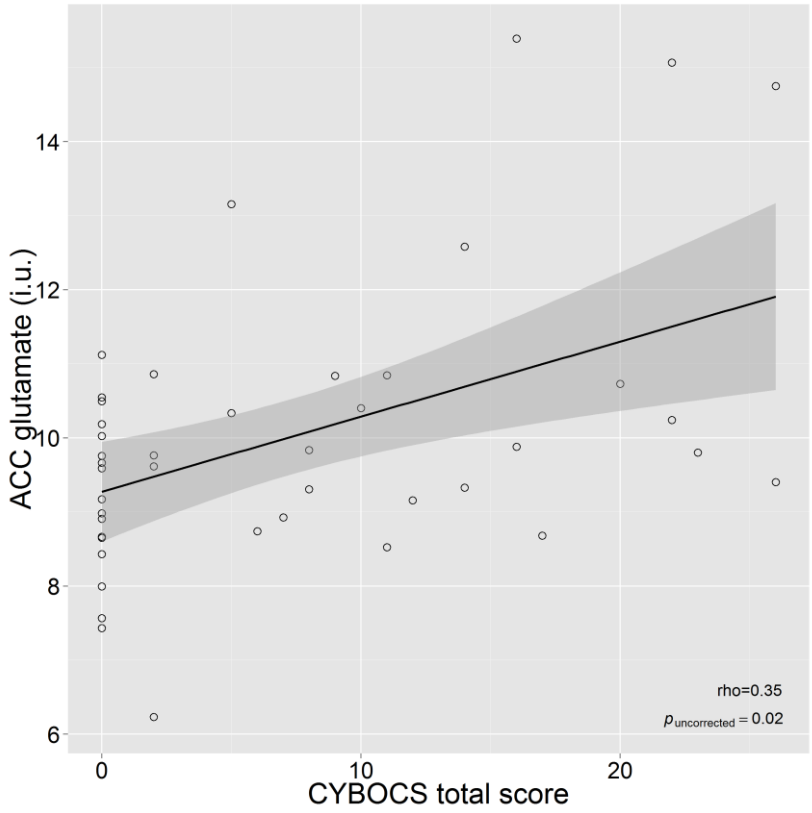


Figure 3. Correlation of ACC glutamate concentration with obsessive-compulsive symptoms (CY-BOCS total score) in participants with TD. ACC, anterior cingulate cortex; CY-BOCS, Children's Yale-Brown Obsessive Compulsive Scale; i.u., Institutional Units; TD, Tourette's disorder. The solid line and its shaded area denote the linear regression fit line and its 95% confidence interval.

## 4. Discussion

This is the first study to investigate glutamate concentrations in both TD and ADHD together. We found no group differences in ACC or left striatal glutamate concentrations. The findings were not confounded by any demographic differences between the groups, spectral quality, medication use or OC-symptoms. Glutamate levels in the ACC correlated with OC-symptoms within the participants with TD (n=44) but did not relate to either tic or ADHD measures of severity.

Only one previous study examined glutamate concentrations in TD by investigating the combined Glx signal in children and adolescents and they were unable to find Glx differences in the brain regions investigated. In corroboration with results presented here (both Glx and glutamate analyses) they found no difference in the putamen but unfortunately did not also examine the ACC.[27] Previous studies did, however, suggest glutamate involvement in TD although the nature of this is yet unclear with both hyper- and hypo-glutamatergic states being hypothesized.[19] For instance, a post-mortem analysis by Anderson and colleagues[23] showed reduced glutamate levels in the GP and substantia nigra of those with TD, while a study investigating serum glutamate concentrations showed increased levels in adult TD patients compared to controls.[49] Our current findings do not support the hypothesis of glutamatergic involvement in the fronto-striatal network in TD.

The current study found no difference in those with ADHD compared to healthy controls or any association between glutamate levels and ADHD severity scores in either the ACC or striatum. Previous studies have been confounded by methodological issues but do appear to show increased Glx levels in both these regions in pediatric ADHD compared to controls,[28] an observation not replicated here, and reduced striatal Glx in adults with ADHD.[50] Associations with symptom severity have been reported previously in adults with ADHD in which negative correlations between inattentive symptoms and glutamate-to-creatine ratios in

the ACC[51] or Glx levels in the basal ganglia[50] were reported. However, these studies were performed in adults, who differ in both ADHD symptoms[2] and glutamate levels[52] compared to children.

Possibly the most interesting finding of the current study, however, is the potential positive correlation between ACC glutamate levels and OC-symptoms within participants with TD. The correlation was present also for both the obsessions and compulsions subscales separately. This suggests the association with ACC glutamate concentration relates to the severity of OC-symptoms irrespective of these dimensions. Elevated ACC glutamate may be associated with cognitive control deficits related to obsessions and compulsions.[53] However, these findings failed to survive correction for multiple comparisons so should be interpreted with caution. Previous literature investigating associations with symptom severity in OCD samples have also shown positive correlations with glutamatergic compounds. For instance, correlations between Glx levels in dorsal and rostral ACC[54] and caudate nucleus[55] and total symptom severity as measured with the Y-BOCS were reported before, although only in adult samples. Our findings should, however, only be interpreted in relation to OC-symptoms within TD. No studies so far have examined glutamatergic compounds in childhood TD and OCD together. Further studies are required to see if the current trend-findings extend to OC-symptoms within pediatric OCD and across other disorders that exhibit similar behaviors, such as ASD. Furthermore, this is a child sample of participants and how these findings relates to OC-symptoms in adult TD will remain unclear until further research is conducted.

The current study ranks among the first to use MRS to investigate brain neurochemical concentrations in TD and is the very first to investigate glutamate concentrations in both TD and ADHD together. However, the study was limited by the small number of TD participants who presented without ADHD/sub-threshold ADHD, these figures are in line with what is expected given the high comorbidity rates of ADHD in TD.[4] It is unlikely that this

significantly hindered the study as our null findings regarding tics and ADHD are supported by the lack of correlations between symptom severity and glutamate levels. These results should not be extrapolated to adults as glutamate concentrations change with age[52] and adult ADHD and TD may well constitute specific presentations of TD and ADHD that persist from childhood. Many participants were medicated, which may alter glutamate levels. However, as the effect of current medication use did not influence glutamate concentrations in either the ACC or striatum medication use was unlikely to have confounded our results. There are several additional factors that may influence glutamatergic signalling such as time of day, sleep, food intake etc. for which we unfortunately could not control.[56,57] Future work is needed to confirm the influence of these factors. Most questionnaires were answered about an un-medicated period, however, a small number of parents opted to answer about a medicated period as they were more familiar with this behavior, this may have led to inconsistent measures of symptom severity in a few cases. Finally, due to low spatial resolution in MRS studies (i.e. large ROIs) it is difficult to determine regionally specific glutamatergic alterations. This is particularly relevant for the striatum ROI, which may contain functionally independent areas with regard to caudate nucleus and putamen. Further investigation should be undertaken to confirm the relation of OC-symptoms and glutamate concentrations in other disorders (OCD, ASD) and also in adult cohorts to determine if the association within TD is limited to children with TD or also present in adult TD.

In conclusion, we found no support for alterations in glutamatergic transmission in the fronto-striatal circuit of children with either ADHD, TD or a combined diagnosis. However, the current study suggests glutamatergic alterations in the ACC in relation to OC-symptoms within children with TD.

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Highlights

- Large pediatric sample of ADHD and TD participants
- 3 Tesla proton MRS utilized to investigate fronto-striatal glutamate concentrations
- No differences in glutamate concentrations in the disorder groups compared with controls
- ACC glutamate concentrations associated with obsessive-compulsive symptoms in TD